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MINI-REVIEW

Genomic adaptation of ethanologenic yeast to biomass conversion inhibitors

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Abstract One major barrier to the economic conversion of biomass to ethanol is inhibitory compounds generated during biomass pretreatment using dilute acid hydrolysis. Major inhibitors such as furfural and 5-hydroxymethylfurfural (HMF) inhibit yeast growth and subsequent fermentation. The ethanologenic yeast *Saccharomyces cerevisiae* demonstrated a dose-dependant inhibition by the inhibitors and has the potential to transform furfural and HMF into less toxic compounds of furfuryl alcohol and 2,5-bis-hydroxymethylfuran (also termed as furan-2,5-dimethanol (FDM)), respectively. For a sustainable and cost-competitive biomass-to-ethanol industry, it is important to develop more tolerant yeast strains that can, in situ, detoxify the inhibitors and produce ethanol. This study summarizes current knowledge and our understanding of the inhibitors furfural and HMF and discusses metabolic conversion pathways of the inhibitors and the yeast genomic expression response to inhibitor stress. Unlike laboratory strains, gene expression response of the ethanologenic yeast to furfural and HMF was not transient, but a continued dynamic process involving multiple genes at the genome level. This suggests that during the lag phase, ethanologenic yeasts undergo a genomic adaptation process in response to the inhibitors. The findings to date provide a strong foundation for future studies on genomic adaptation and manipulation of yeast to aid more robust strain design and development.

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Introduction

As interest in alternative energy sources rises, the concept of agriculture as an energy producer has become increasingly attractive (Outlaw et al. 2005). Renewable biomass, including lignocellulosic materials and agricultural residues, are low-cost materials for bioethanol production (Bothast and Saha 1997; Wheals et al. 1999; Zaldivar et al. 2001). One major barrier of biomass conversion to ethanol is inhibitory compounds generated during biomass pretreatment using dilute acid hydrolysis, which interfere with microbial growth and subsequent fermentation. Furfural and 5-hydroxymethylfurfural (HMF) are major inhibitors commonly recognized from the biomass pretreatment. The genetic mechanisms involved in stress tolerance, such as those caused by furfural and HMF, are not understood. Few yeast strains tolerant to inhibitors are available. The development of stress-tolerant ethanologenic yeast is one of the significant challenges for cost-competitive bioethanol production. This article summarizes knowledge of the inhibitors furfural and HMF and discusses the potential for tolerant yeast strain improvement and genomic adaptation of ethanologenic yeast to cope with furfural and HMF stress to aid future strain design and development.

Inhibitors

Furfural and HMF derived from biomass pretreatment

For economic reasons, dilute acid hydrolysis is commonly used in biomass degradation for enzymatic saccharification

and fermentation (Bothast and Saha 1997; Saha 2003). However, numerous other compounds are generated by this pretreatment, many of which inhibit microbial growth and metabolism. More than 100 compounds have been detected to have potential inhibitory effects on microbial fermentation (Luo et al. 2002; Klinke et al. 2004). Major inhibitors produced from hydrolysis of lignocellulosic materials can be assigned to three groups that include furan derivatives, weak acid, and phenolic compounds (Palmqvist and Hahn-Hägerdal 2000). Furfural and HMF are considered to be the most potent inhibitors to yeast growth and fermentation (Chung and Lee 1985; Olsson and Hahn-Hägerbal 1996; Taherzadeh et al. 2000a).

During biomass degradation by dilute acid treatment, furfural and HMF are derived from the dehydration of pentoses and hexoses, respectively (Dunlop 1948; Antal et al. 1990, 1991; Larsson et al. 1999; Lewkowski 2001). These compounds reduce enzymatic and biological activities, break down DNA, and inhibit protein and RNA synthesis (Sanchez and Bautista 1988; Khan and Hadi 1994; Modig et al. 2002). Yeasts can be killed by the inhibitory complex even at low concentrations (Liu et al. 2004). Most yeasts, including industrial strains, are susceptible to the complexes associated from dilute acid hydrolysis pretreatment (Palmqvist et al. 1999; Taherzadeh et al. 2000a; Martin and Jonsson 2003; Liu et al. 2004). To facilitate fermentation processes, additional remediation treatments, including physical, chemical, or biochemical detoxification procedures, are often required to remove these inhibitory compounds. However, these additional steps add cost and complexity to the process and generate extra waste products.

Metabolic conversion pathways of the inhibitors

Furfural conversion to furfuryl alcohol by yeasts has been well established (Morimoto and Murakami 1967; Villa et al. 1992; Mohsenzadeh et al. 1998). It is suggested that furfural is first converted to furfuryl alcohol and further reduced to pyromucic acid (Nemirovskii and Kostenko 1991). Furfural can also break down to form formic acid (Palmqvist and Hahn-Hägerdal 2000). Under anaerobic conditions, furfural is reduced to furfuryl alcohol by nicotinamide adenine dinucleotide (NADH)-dependent dehydrogenases by yeasts (Palmqvist et al. 1999). In the presence of furfural, the ATP level is low and cell replication is limited. Glycerol formation is reduced. Furfural has been quantitatively characterized as an electron acceptor (Wahlbom and Hahn-Hägerdal 2002). Shortage of NADH was observed in the presence of furfural. It appears that furfural reduction competes for NADH because the acetaldehyde reduction process needs NADH to regenerate NAD^+. As a result, furfural could cause the accumulation of acetaldehyde and a delay of acetate and ethanol production. Furfural can also be reduced by nicotinamide adenine dinucleotide phosphate (NADPH)-dependent alcohol dehydrogenase encoded by *ADH6*, which has a broad specificity of substrate (Larroy et al. 2002; Petersson et al. 2006). Xylitol excretion was reduced during xylose fermentation when furfural was added into the medium (Wahbom and Hahn-Hägerdal 2002). Reduced furfural tolerance was observed for selective deletion mutants of genes in the pentose phosphate pathway (Gorsich et al. 2005). This suggests a potential relationship between these genes and furfural reduction.

Unlike the well studied furfural, knowledge on HMF conversion is limited because there is not a readily available commercial source for an HMF conversion product. However, HMF and furfural are chemically related and both have a furan ring and an aldehyde group in their chemical structures. Following the furfural conversion route, HMF has been assumed to convert into HMF alcohol (Nemirovskii et al. 1989). Although most studies have assumed this conversion, HMF alcohol has not been isolated from cultures, nor has its structure been identified. Proposed general reduction models were not consistent with experimental observations (Sanchez and Bautista 1988; Nemirovskii et al. 1989; Wahbom and Hahn-Hägerdal 2002). The lack of an HMF conversion product has limited interpretations and progress of studies in this area. The mechanism of HMF metabolism and its involvement in the fermentation pathway need to be clarified.

HMF was detectable at absorbance at 282 nm, but it was undetectable 48 h after incubation at 30 mM in a yeast culture (Liu et al. 2004). Instead, a new peak of the HMF-associated product was detected at 222 nm (Fig. 1a,b). Based on consistent observations, an end metabolic conversion product of HMF by the yeast was isolated and purified from cell-free culture supernatant. The chemical structure of the metabolite was characterized using mass and NMR spectra analysis. The signals for the aldehyde proton and the asymmetric spectra of HMF were absent when the purified HMF-conversion product was analyzed using NMR. The NMR spectra are consistent with that of a symmetrical molecule with a furan ring. This molecule was identified as 2,5-bis-hydroxymethylfuran [also termed as furan-2,5-dimethanol (FDM)] (Liu et al. 2004). The chemical structure of the metabolite was identified as a compound with a composition of $C_6H_8O_3$ and a molecular weight of 128 (Fig. 1c). This rigorous chemical identification of FDM is the first report of the chemical structure of the metabolic reduction product of HMF by yeast. It clarifies the existing literature and provides basic evidence for a fermentation pathway that yeasts can utilize for response in the presence of HMF. It was further demonstrated that this transformation is a yeast-catalyzed bio-

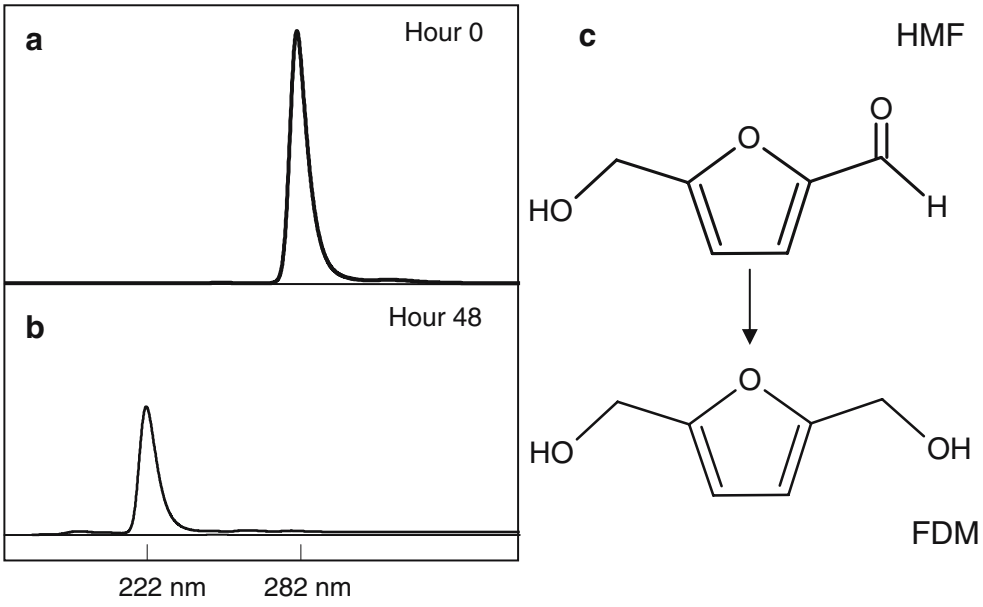

Fig. 1 HMF conversion to FDM (based on Liu et al. 2004). Reverse-phase high-performance liquid chromatography chromatogram of culture supernatant from a defined medium amended with HMF for ethanologenic yeast *S. cerevisiae* showing HMF detection by UV absorbance at 282 nm at 0 h (**a**) but not detectable 48 h after incubation (**b**). A new peak at 222 nm previously not observed was detected (**b**) and identified as 2,5-bis-hydroxymethylfuran (also termed as FDM) with a chemical composition of $C_6H_8O_3$ (**c**) and molecular mass ion of 128 by GC–MS and NMR

transformation process (Liu et al. 2005). For example, in a culture medium amended with 120 mM of HMF, no cell growth was observed and no HMF conversion occurred. As measured by high-performance liquid chromatography, glucose and HMF in the medium remained at the original concentrations 5 days after incubation.

Unlike furfural reduction, which requires NADH, HMF has a different cofactor preference with NADPH needed for its reduction (Wahbom and Hahn-Hägerdal 2002). This suggested a difference in relationships of reducing enzymes involved and inhibition mechanisms for the two inhibitors. HMF conversion could compete with biomass synthesis and cell growth because NADPH is required for the synthesis of amino acids and nucleotides. It is well recognized that HMF can persist for a longer period of time while furfural is rapidly depleted in a yeast culture (Larsson et al. 1999; Taherzadeh et al. 2000a; Wahbom and Hahn-Hägerdal 2002; Liu et al. 2004). Any adaptation strategy has to be specific to HMF or furfural to accomplish higher levels of tolerance (Liu unpublished data). Therefore, the metabolic conversion pathway of furfural and HMF involved in ethanol fermentation pathway may differ, although similar enzymatic reduction reactions can be involved. Variation of cofactor preference of NADH and NADPH for HMF reduction was observed between two different strains of *Saccharomyces cerevisiae* (Nilsson et al. 2005). A general model of the furfural and HMF conversion pathways relative to glycolysis and ethanol fermentation is proposed (Fig. 2). In this prototype pathway, the main routes of the reduction of furfural and HMF compete for cofactor NADH, and NADPH, respectively. Reduction pathways involved in recently reported reductase with different cofactor preferences for each inhibitor are illustrated in dotted lines. NAD^+ is regenerated from NADH to enable continued glycolysis. Furfural competes for NADH during acetaldehyde reduction. In case HMF conversion also requires NADH, it adds additional pressure to the competition of the limited NADH. Similarly, synergetic competition of NADPH affects biosynthesis pathways. At higher concentrations, these substrates can dominate the competition. As a result, the metabolic process can be significantly altered and delayed in the presence of the inhibitors. In the presence of the inhibitors, glucose was not consumed until appropriate furfural and/or HMF reduction levels were reached (Liu et al. 2004). This could partially explain the delayed glycolysis and the synergetic repression to yeast demonstrated by the combined treatment of furfural and HMF as previously described by Liu et al. (2004). However, variations of cofactor preference for furfural and HMF among different strains need to be confirmed. Nonetheless, the presence of furfural and HMF apparently affect cellular redox balance.

Yeast adaptation to the inhibitors

Microbial performance is the key for cost-efficient improvement

The economics of fermentation-based bioprocesses rely extensively on the performance of microbial biocatalysts in industrial application. This is true for bioethanol production as well. Improving microbial performance is the key to future commercial sustainability of the biomass-to-ethanol industry. Fermentation is among the oldest microbial

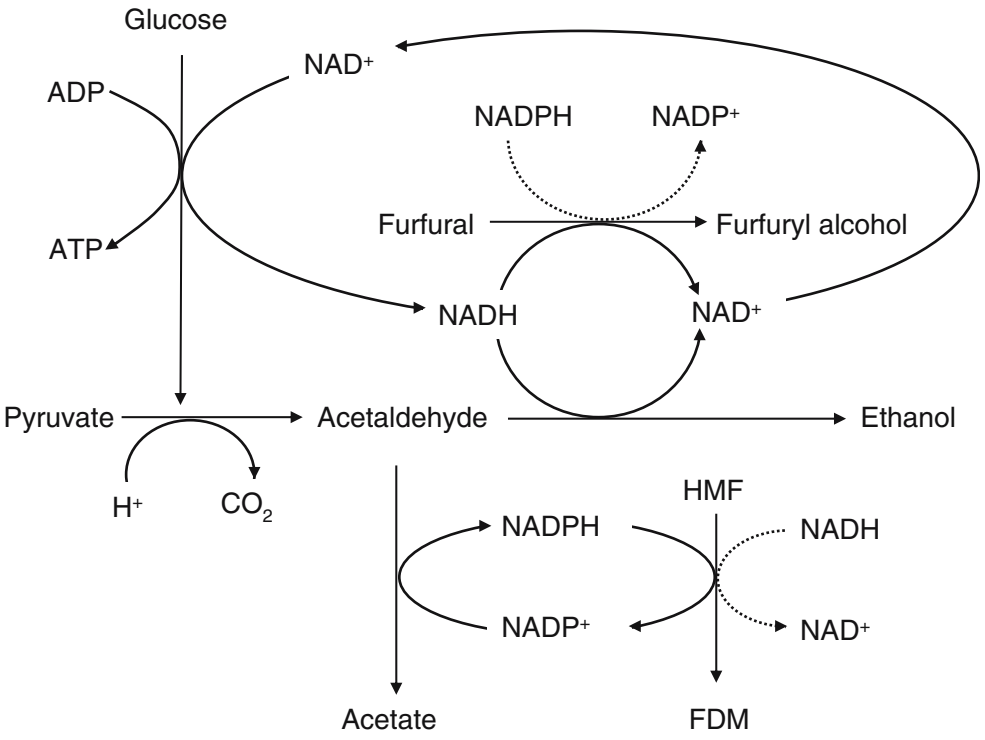

Fig. 2 The furfural and HMF conversion pathways. A schematic diagram shows furfural conversion into furfuryl alcohol and HMF into FDM relative to glycolysis and ethanol fermentation for the ethanologenic yeast (see text for detailed description)

applications in human history. Although a tremendous amount of knowledge has been accumulated through years of experience and development of modern technology, many alternative fermentation processes remain unknown. Continued research and development, specifically on stress tolerance mechanisms involved in the biomass-to-ethanol conversion process, are needed.

Genetically manipulated yeast strains have shown enhanced functions for ethanol fermentation through improved utilization of starch, lactose, and xylose, as well as enzyme production (Ho et al. 1998; Jeffries and Shi 1999; Ostergaard et al. 2000; Hahn-Hägerdal et al. 2001). The development of genetically engineered strains with greater inhibitor tolerance, especially to furfural and HMF, is a promising alternative means to the traditional inhibitor remediation steps (Liu and Slininger 2005; Liu et al. 2005). However, the development of such strains is hindered due to a lack of understanding of the basic mechanisms underlying stress tolerance in ethanologenic yeast. The fast life cycle and genetic diversity of ethanologenic yeasts are invaluable resources for strain improvement. Efficient utilization of these characteristics will lead to more cost-effective fermentation and process in the future.

Dose-dependent response of cell culture

Using a series of concentrations of inhibitors incorporated into a synthetic medium, a dose-dependent response of ethanologenic yeast to furfural and HMF at concentrations from 10 to 120 mM was characterized (Liu et al. 2004). Given a tolerable concentration of furfural and HMF individually or in combination, such as 10 or 30 mM, yeast strains were able to recover from a prolonged lag phase during the initial stage of the incubation. The lag phase lasted from a few hours to several days depending upon the concentration of the inhibitors in the cultures and varied strains. Once the cell growth recovered, cultures inoculated with sublethal doses of the inhibitors were able to consume glucose and, thereafter, produce ethanol. This demonstrated a clear dose-dependent inhibition of the yeast by furfural and HMF.

The duration of the lag phase may be interpreted as a measurement of varied levels of tolerance to furfural and HMF. No metabolic activity was detected from cultures of cells repressed to death. Only the adapted or surviving cells functioned further to produce ethanol. This interpretation further suggests that some yeast strains have more effective mechanisms to withstand these inhibitors than others do. The prolonged lag phase before the recovery of the cell growth could reflect a genetic response and a shift in physiology of the cells adapting to the chemical stress. Enzymatic induction was suggested during the lag phase (Kang and Okada 1973; Liu and Slininger 2005, 2006). Important metabolic enzymes including alcohol dehydrogenase, aldehyde dehydrogenase, and pyruvate dehydrogenase have been reported to be inhibited in vitro by furfural and HMF (Modig et al. 2002). The persistence of specific altered gene expression over time supports the hypothesis that yeasts are stimulated to undergo an adaptation process

during the lag phase in response to HMF (Liu and Slininger 2006).

Enhanced biotransformation and tolerance

The adaptation of *S. cerevisiae* to furfural and HMF has been observed and applied to overcome inhibitory effects using increased yeast inoculum levels, increased biomass, or in fed-batch mode fermentation (Banerjee et al. 1981; Chung and Lee 1985; Villa et al. 1992; Taherzadeh et al. 2000b). An adapted *Pichia stipitis* was reported to have improved ethanol production from hemicellulose hydrolysate (Nigam 2001). Based on the dose-dependent yeast response to furfural and HMF, strains more tolerant to furfural or HMF were generated using a directed adaptation method (Liu et al. 2005). The tolerant strains had nearly normal cell growth in contrast to a prolonged lag phase in the wild type under the challenge of the inhibitors (Fig. 3). Recently, a newly developed strain showed tolerance to both furfural and HMF and completed ethanol fermentation in 48 h (Liu unpublished data). It did not require a prebuild biomass but functioned as an initial inoculum to establish a culture and complete the fermentation. The tolerant strain showed significantly enhanced biotransformation to convert furfural into furfuryl alcohol and HMF into FDM and produced a normal yield of ethanol. In contrast, a normal control strain failed to establish a culture in the presence of the inhibitors 48 h after inoculation. This indicated a qualitative change derived from evolution of the quantitative adaptation, a genetic alteration in cell response to the inhibitors. Thus, it is possible to in situ detoxify furfural and HMF generated by economic dilute acid hydrolysis for cost-competitive bioethanol conversion using tolerant yeast strains. Numerous genes encoding varied reductases were significantly induced and were found to be responsible for HMF conversion into FDM (Liu et al. 2004; Liu and Slininger 2006). HMF reduction activity by *ADH6* encoding enzyme was recently reported (Petersson et al. 2006).

Apparently, yeast has internal genetic potential for further improvement to tolerate inhibitors such as furfural and HMF. The adaptation approach can be an alternative means to improve microbial strain performance. Such adapted strains could be efficiently used for further genetic manipulation. Further study in this area is expected for new strain development. The currently available strains are valuable resources, although they may not be able to withstand multiple inhibitor complexes, such as those in crude biomass hydrolysates, and will need further improvement. Nonetheless, single and double inhibitor-tolerant strains are necessary to dissect the mechanisms of stress tolerance to multiple inhibitor complexes.

Towards genomic studies

Understanding mechanisms of tolerance using functional genomics

Single-gene studies have contributed significantly to our understanding of gene functions and gene regulatory rules in the past 50 years. However, a biological process often cannot be explained by a single gene function. For example, xylose utilization genes can be incorporated into nonxylose-utilizing *S. cerevisiae* to enable the yeast to convert xylose (Ho et al. 1998). Because the genomic regulatory mechanism is not clear, the improved yeast can consume xylose when the preferred glucose is nearly exhausted rather than utilize both sugars simultaneously as expected. Significant genomic regulatory elements are needed for efficient genetic manipulation. Life, as a complex dynamic system, functions under an integrated control program rather than as an isolated event. Considering that thousands of genes in a genome are required to maintain a living yeast system, significant gene alteration impacts the responses of other genes in the system. To understand mechanisms of stress tolerance to furfural and

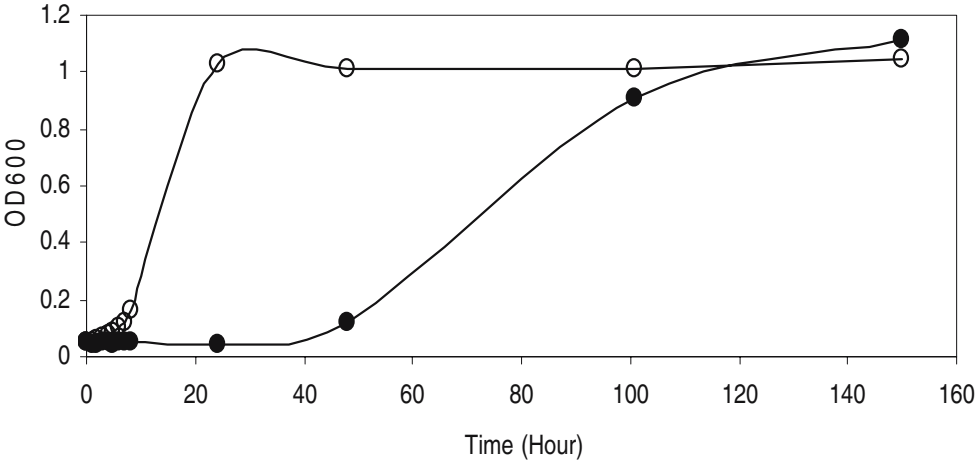

Fig. 3 The ethanologenic yeast culture adaptation to HMF. Cell growth of an adapted and more tolerant strains of ethanologenic yeast *S. cerevisiae* (*open circles*) and the wild-type parental strain (*closed circles*) on a defined medium under HMF stress condition for a complete fermentation course showing a lag phase of an extended delay of cell growth for the wild type compared with the normal growth rate for the adapted strain

HMF, it is necessary to identify key gene functions, gene interaction networks, and regulatory elements involved at the genome level. By using integrated gene microarray technology, we can study global transcriptome profiling and gain insight into dynamic living responses to furfural and HMF stress.

Yeasts live in ever-changing environments and need to constantly adapt to external stimuli for survival. As documented in numerous reports, yeast adaptation to stress conditions is common and accomplished via a variety of molecular mechanisms (Gasch and Werner-Washburne 2002; Erasmus et al. 2003). As a model organism, laboratory strains of yeasts have been studied intensively by genomic expression profiling to varied environmental stimulants (Gasch et al. 2000; Causton et al. 2001; Brejning et al. 2003; Zhang et al. 2003; Lucau-Danila et al. 2005). Common stress-tolerant genes are reported and the transient expression response to stimuli is common. Genome expression and transcriptome dynamics of some industrial yeasts to environmental stress and other fermentation stress conditions including HMF are also studied (Chen et al. 2003; Erasmus et al. 2003; James et al. 2003; Devantier et al. 2005; Liu and Slininger 2006). Unlike the transient responses of laboratory stains, industrial strains showed more persistent expression patterns. However, systematic information on inhibitory stress tolerance involved in the bioethanol conversion process at the genome level is not yet available. Due to the heterogeneity of experimental conditions and a lack of common quality control for multiple microarray experiments, it is impossible to make comparisons among these results. Currently, an integrated functional genomic approach is being undertaken to study furfural and HMF stress tolerance involved in bioethanol conversion and to develop more tolerant strains (Liu and Slininger 2005).

Quality control issues for gene expression analysis

For reliability and reproducibility of microarray expression data, one cannot overestimate the significance of the proper application of quality controls. The quality control has been a very important issue since the emergence of high-throughput gene expression technology (Schena et al. 1995; Brazma et al. 2001; Badiee et al. 2003). It has drawn ever-increasing attention with concerns of application of expression data (Baker et al. 2005; Bammler et al. 2005; Larkin et al. 2005). The need of standard controls across different platforms of gene expression analysis has been recognized (Dallas et al. 2005; Etienne et al. 2005; External RNA Controls Consortium 2005; Irizarry et al. 2005). A set of universal external RNA quality controls was developed specifically for microbial gene expression analysis across different platforms of microarray and real-time quantitative reverse transcriptase (RT)-PCR (Liu and Slininger submitted). Six species of exogenous nucleotides were used. The DNA sequences of these control genes were compared with those in the microbial gene sequence database (Peterson et al. 2001). The selected control genes were not homologous or had no similarity to the yeast genome and bacterial system, and therefore, avoided interference with microbial gene expression signals. The linearity of signal intensity of the control genes allowed them to serve as a quantitative calibration reference for gene expression measurements. Using these quality controls, a coefficient of variance can be calculated and analysis of variance can be applied (Kerr and Churchill 2001; Churchill 2004). Unlike housekeeping genes usually affected by environmental conditions, these controls demonstrated consistency in mRNA detection independent from environmental factors. Such quality control measurements provide a normalization reference, allow an estimate of variation for microarray experiments, reduce variability, and increase reliability and reproducibility of the microarray data. Application of universal quality controls will allow confirmation and comparison of data obtained from different microarray experiments and platforms. To generate quality data from microarray experiments, it is strongly recommended that the complete length of cDNA populations of labeled probes be evaluated using a microgel or slide gel electrophoresis system (Lage et al. 2002; Liu and Slininger 2005). Such a quality control measurement cannot be substituted by quantitative measurements of the probe using a spectrophotometer.

Genomic expression response to the inhibitors

Genomic expression profiles of the wild-type ethanologenic yeast *S. cerevisiae* in response to furfural and HMF stress conditions were investigated. Yeast genes responded immediately at least 10 min after exposure to the inhibitor (Liu and Slininger 2006). The expression levels of several hundred genes were significantly different for the yeast under HMF-challenged conditions compared with those of an untreated normal control. These genes demonstrated significant differential expression patterns during a lag phase under HMF stress and are more likely to be responsible in the adaptation for survival to cope with the HMF stress (Fig. 4). Among those significantly induced genes, members of the pleiotropic drug resistance gene family were assumed to play an important role in coping with the inhibitor stress for cell survival. Key functional candidate genes were identified. Unlike the transient changes reported for laboratory strains, constant functional mRNA expression was observed for the ethanologenic yeast in response to the HMF stress. During the lag phase, some genes showed continued enhanced or repressed expression, while others demonstrated significant dynamics

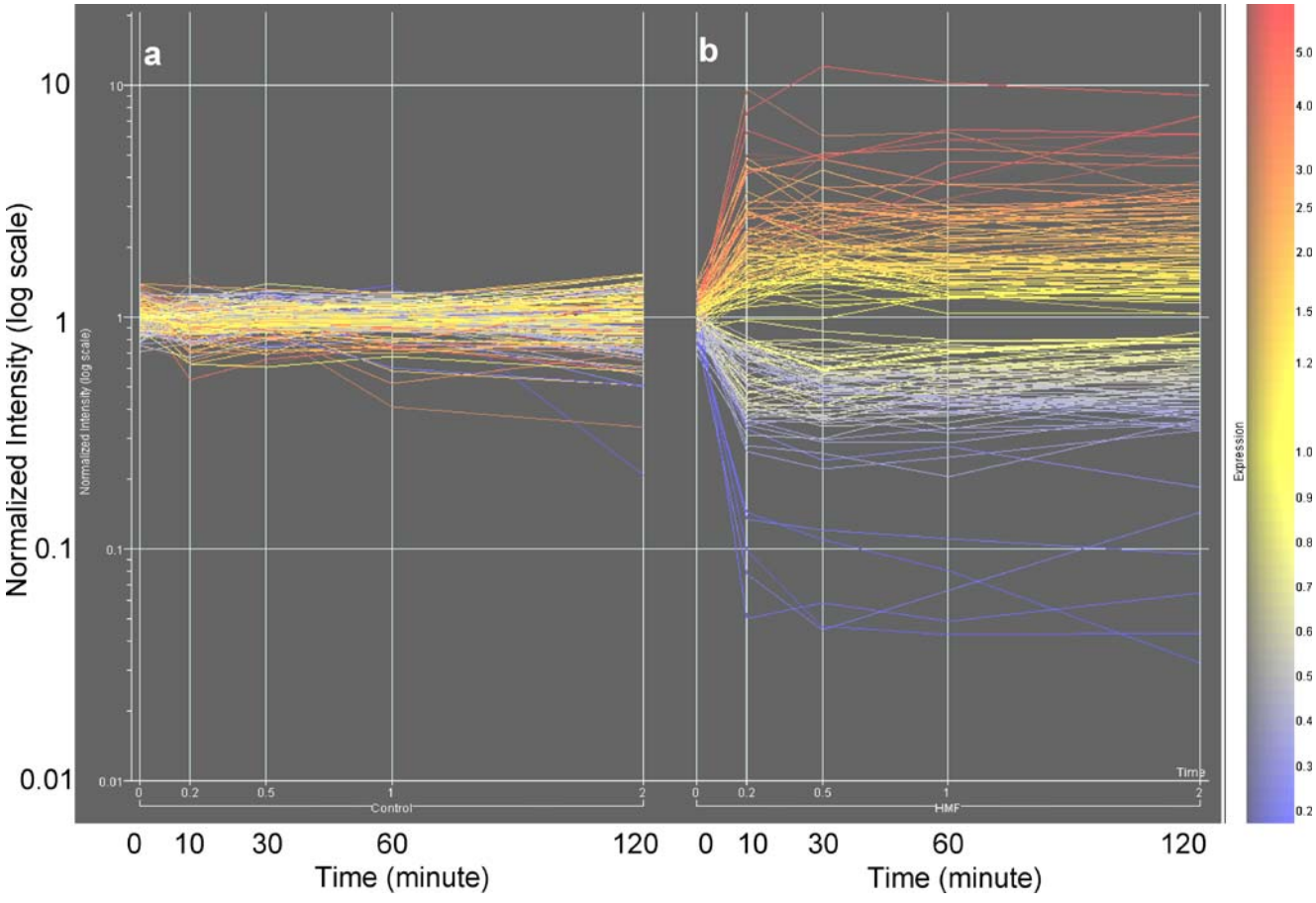

Fig. 4 The genomic adaptation to HMF stress. Expression of selected genes of ethanologenic yeast *S. cerevisiae* under a normal control condition (**a**) and HMF stress condition (**b**) from 0, 10, 30, 60, and 120 min after the treatment showing significantly induced (*blue*) and repressed (*red*) mRNA expression caused by the HMF stress on a defined medium. Yellow color indicates mRNA equally expressed under different conditions. Varied colors between yellow and red, or yellow and blue, as shown in a *colored bar* on the far right, indicate varied quantitative measurements of mRNA expression levels in a log scale

of reversed expression (Liu and Slininger 2006). Such expression patterns are significantly different from those of a control. The HMF-treated yeast resumed dramatic fermentative metabolic activity and produced a normal yield of ethanol. This suggests a genetic adaptation response of the yeast to HMF stress during the lag phase. Confirmation of differentially expressed genes by the inhibitor stress in the lag phase is under investigation. Some of significantly induced expression by furfural and HMF were confirmed using real-time quantitative RT-PCR (Liu unpublished data). Genes were identified in categories of biological processes, cellular components, and molecular function. Among which, some genes appeared to be HMF-specific while others shared functions with those in a core set of common stress genes. However, the interpretation of some genes was limited by incomplete annotations or lack of known functions. Computation inference of gene regulatory networks has been incorporated and biological experimental conformation is needed. Studies on mechanisms of the tolerance using the tolerant strain are currently underway.

Conclusions

The isolation and identification of HMF metabolic conversion end product as FDM has clarified existing literature and provides a basis for metabolic profiling studies of yeast on inhibitor stress tolerance. The dose-dependent inhibition of the ethanologenic yeast allowed its potential adaptation to the inhibitors to transform furfural and HMF into less toxic compounds of furfuryl alcohol and FDM, respectively. A genomic approach is needed for efficient improvement of ethanologenic yeast performance. For high-throughput genomic expression studies, the proper application of quality control measurements is critical to ensure the reliability

and reproducibility of expression data and allows data confirmation and comparison. Gene expression responses of the ethanologenic yeast to furfural and HMF stress during the fermentation were not transient. In fact, the yeast adaptation to furfural and HMF was a continued dynamic process involving multiple genes at the genome level. Based on these observations, this study proposed a concept of genomic adaptation by the ethanologenic yeasts to furfural and HMF stress, specifically as was described during the lag phase. With the aid of the comprehensive and updated yeast database, we can readily explore global transcriptome profiling of the ethanologenic yeast and provide additional insights into the complexity of adaptation to the inhibitor stress. However, a great deal of knowledge remains unknown. Among the significant genes involved in the adaptation, some genes were found to have limited annotation of defined functions. Challenges remain to assign complete functions, draw meaningful conclusions from the complex relationships, and assess biological confirmations of gene regulatory networks. Global transcriptome profiling of the tolerant strain is under investigation. Key function genes and relevant regulatory components responsible for the biotransformation and detoxification of the inhibitor will be characterized. Bioinformatics and computation biology have been incorporated into the program. The genomic mechanism of stress tolerance to furfural, HMF, and the inhibitory complex involved in bioethanol conversion will be elucidated to aid more robust strain design and development in the future.

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